

SEQUENCE LISTING

<110> Lydiate, Derek  
Hannoufa, Abdelali  
Bate, Nicholas  
Hegedus, Dwayne

<120> Repressor Mediated Selection Strategies

<130> 11089.0003.NPUS01

<150> 60/416,369

<151> 2002-10-03

<160> 61

<170> PatentIn version 3.1

<210> 1

<211> 472

<212> DNA

<213> artificial

<220>

<223> Synthetic Ros optimized for plant expression

<400> 1

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ctggacttat ctctgatggt catactgcac tttctggaac atctgctcct gcttctgttg	180
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atcactctat gactccagag gagtatagag agaagtggga tcttcctggt gattacccta	360
tggttgctcc tgcttacgct gaggtcgtt ctcgtctcgc taaggagatg ggtctcggtc	420
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<210> 2

<211> 678

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<220>

<223> Synthetic Tet optimized for plant expression

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gagcagccta cgttgtagtg gcatgttaag aacaagcggg ctttgctcga cgccctcgcg	180
attgagatgt tagacaggca ccatactcac ttctgccctc tcgaagggga gagctggcaa	240

gatttcctcc gtaacaacgc taagtccttc agatgtgctc tcctatccca tcgcgacgga 300  
gcaaaagtcc atctgggtac acggcctaca gagaaac agt atgagactct cgaaaatcaa 360  
ctggcctttc tgtgccaaca gggtttctca ctagagaatg cgctttacgc actctcagct 420  
gtggggcatt ttactcttgg ttgcgttttg gaggatcaag agcatcaagt cgctaaggaa 480  
gagagggaaa cacctactac tgatagtatg cgcgcacttc ttcgacaagc catcgaactt 540  
tttgatcacc aggggtgcaga gccagccttc ttgttcggcc ttgaattgat catatgcgga 600  
ttggaaaagc agcttaaagt tgaatcgggg tctcttaagc caaaaaagaa gcgtaaggtc 660  
tgacttaagt gaatcgat 678

<210> 3  
<211> 149  
<212> PRT  
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<220>  
<223> Synthetic Ros

<400> 3

Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu  
1 5 10 15

Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val  
20 25 30

Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly  
35 40 45

Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys P ro  
50 55 60

Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu  
65 70 75 80

Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu T hr Thr His  
85 90 95

His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Val  
100 105 110

Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu A la Arg Ser Arg Leu  
115 120 125

Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg Pro Lys  
130 135 140

Lys Lys Arg Lys Val  
145

<210> 4  
<211> 216  
<212> PRT  
<213> Artificial

<220>  
<223> Synthetic Tet

<400> 4

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu  
1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln  
20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys  
35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His  
50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg  
65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly  
85 90 95

Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr  
100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu  
115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys  
130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr  
145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu  
165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu  
 180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser Leu  
 195 200 205

Lys Pro Lys Lys Lys Arg Lys Val  
 210 215

<210> 5  
 <211> 24  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Actin2 promoter sense primer

<400> 5  
 aagcttatgt atgcaagagt cagc 24

<210> 6  
 <211> 24  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Actin2 promoter anti -sense primer

<400> 6  
 ttgactagta tcagcctcag ccat 24

<210> 7  
 <211> 27  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ros sense primer

<400> 7  
 gcggatccga tgacggaaac tgcatac 27

<210> 8  
 <211> 25  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ros anti -sense primer

<400> 8  
 gcaagcttca acggttcgcc ttgcg 25

<210> 9  
<211> 36  
<212> DNA  
<213> Artificial

<220>  
<223> iaaH sense primer

<400> 9  
tgcggatgca taagcttgct gacattgcta gaaaag 36

<210> 10  
<211> 26  
<212> DNA  
<213> Artificial

<220>  
<223> iaaH anti-sense primer

<400> 10  
cggggatcct ttcagggcca tttcag 26

<210> 11  
<211> 43  
<212> DNA  
<213> Artificial

<220>  
<223> Tet-FI primer

<400> 11  
gatcactcta tcagtgatag agtgaactct atcagtgata gag 43

<210> 12  
<211> 41  
<212> DNA  
<213> Artificial

<220>  
<223> Tet-RI primer

<400> 12  
cgctctatca ctgatagagt tcactctatc actgatagag t 41

<210> 13  
<211> 26  
<212> DNA  
<213> Artificial

<220>  
<223> iaaH ORF sense primer

<400> 13  
gctctagaat ggtgccatt acctcg 26

<210> 14  
<211> 26  
<212> DNA  
<213> Artificial  
  
<220>  
<223> iaaH ORF anti -sense primer  
  
<400> 14  
gcgagctcaw atggcttytt cyaatg 26  
  
<210> 15  
<211> 59  
<212> DNA  
<213> Artificial  
  
<220>  
<223> Ros -OP1  
  
<400> 15  
gatcctatat ttcaatttta ttgtaatata gctatatttc aattttattg taatataat 59  
  
<210> 16  
<211> 57  
<212> DNA  
<213> Artificial  
  
<220>  
<223> Ros -OP2  
  
<400> 16  
cgattatatt acaataaaat t gaaatatag ctatattaca ataaaattga aatatag 57  
  
<210> 17  
<211> 25  
<212> DNA  
<213> Agrobacterium tumefaciens  
  
<400> 17  
tatatttcaa ttttattgta atata 25  
  
<210> 18  
<211> 27  
<212> DNA  
<213> Agrobacterium tumefaciens  
  
<400> 18  
tataattaaa atattaactg tcgcatt 27  
  
<210> 19  
<211> 429  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 19  
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 gatgttcata cggcactcag cggaacatcg gcaccggcat cggtggcgggt caatgttgaa 180  
 aagcagaagc ctgctgtgtc gggtcgcaag tcgggttcagg acgatcatat cgtctgtttg 240  
 gaatgtggtg gtcggttcaa gtcgctcaaa cgccacctga cgacgcatca cagcatgacg 300  
 ccggaagaat atcgcgaaaa atgggatctg ccggtcgatt atccgatggt tgctcccgcc 360  
 tatgccgaag cccgttcgcg gtcgccaag gaaatgggtc tcggtcagcg ccgcaaggcg 420  
 aaccgttga 429

<210> 20  
 <211> 624  
 <212> DNA  
 <213> escherichia coli

<400> 20  
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 ggaatcgaag gcctaacaac ccgtaaactt gcgcagaagc tcggggtaga gcagcctaca 120  
 ttgtattggc atgtaaaaaa taagcggggc ctgctcgacg cgtagccat tgagatgtta 180  
 gataggcacc atactcactt ttgcccttta gaaggggaaa gctggcaaga ttttttacgt 240  
 aataacgcta aaagtttttag atgtgcttta ctaagtcacg gcgatggagc aaaagtacat 300  
 ttaggtacac ggcctacaga aaaacagtat gaaactctcg aaaatcaatt agccttttta 360  
 tgccaacaag gtttttcact agagaatgca ttatatgcac tcagcgtgtg ggggcatttt 420  
 acttttaggtt gcgtattgga agatcaagag catcaagtcg ctaaagaaga aagggaaca 480  
 cctactactg atagtatgcc gccattatta cgacaagcta tcgaattatt tgatcaccaa 540  
 ggtgcagagc cagccttctt attcggcctt gaatt gatca tatgcggatt agaaaaacaa 600  
 cttaaattgtg aaagtgggtc ttaa 624

<210> 21  
 <211> 142  
 <212> PRT  
 <213> Agrobacterium tumefaciens

<400> 21

Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu  
 1 5 10 15

Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val  
 20 25 30

Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly  
35 40 45

Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys Pro  
50 55 60

Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu  
65 70 75 80

Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu Thr Thr His  
85 90 95

His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Val  
100 105 110

Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu  
115 120 125

Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg  
130 135 140

<210> 22  
<211> 207  
<212> PRT  
<213> Escherichia coli

<400> 22

Met Ser Arg Leu Asp Lys Ser Lys Val Ile Asn Ser Ala Leu Glu Leu  
1 5 10 15

Leu Asn Glu Val Gly Ile Glu Gly Leu Thr Thr Arg Lys Leu Ala Gln  
20 25 30

Lys Leu Gly Val Glu Gln Pro Thr Leu Tyr Trp His Val Lys Asn Lys  
35 40 45

Arg Ala Leu Leu Asp Ala Leu Ala Ile Glu Met Leu Asp Arg His His  
50 55 60

Thr His Phe Cys Pro Leu Glu Gly Glu Ser Trp Gln Asp Phe Leu Arg  
65 70 75 80

Asn Asn Ala Lys Ser Phe Arg Cys Ala Leu Leu Ser His Arg Asp Gly  
85 90 95



Ala Lys Val His Leu Gly Thr Arg Pro Thr Glu Lys Gln Tyr Glu Thr  
 100 105 110

Leu Glu Asn Gln Leu Ala Phe Leu Cys Gln Gln Gly Phe Ser Leu Glu  
 115 120 125

Asn Ala Leu Tyr Ala Leu Ser Ala Val Gly His Phe Thr Leu Gly Cys  
 130 135 140

Val Leu Glu Asp Gln Glu His Gln Val Ala Lys Glu Glu Arg Glu Thr  
 145 150 155 160

Pro Thr Thr Asp Ser Met Pro Pro Leu Leu Arg Gln Ala Ile Glu Leu  
 165 170 175

Phe Asp His Gln Gly Ala Glu Pro Ala Phe Leu Phe Gly Leu Glu Leu  
 180 185 190

Ile Ile Cys Gly Leu Glu Lys Gln Leu Lys Cys Glu Ser Gly Ser  
 195 200 205

<210> 23  
 <211> 10  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Consensus Ros operator sequence

<400> 23  
 watdhwkmar

10

<210> 24  
 <211> 7  
 <212> PRT  
 <213> SV40

<400> 24

Pro Lys Lys Lys Arg Lys Val  
 1 5

<210> 25  
 <211> 109  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ros-OPDS

<400> 25  
atctccactg acgtaaggga tgacgcacaa tcccactatc cttcgcaaga cccttcctct 60  
atataatata tttcaatttt attgtaatat aacacggggg actctagag 109

<210> 26  
<211> 113  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPDA

<400> 26  
gatcctctag agtccccgt gttatattac aataaaattg aaatatatta tatagaggaa 60  
gggtcttgcg aaggatagtg ggattgtgcy tcattccctta cgtcagtga gat 113

<210> 27  
<211> 138  
<212> DNA  
<213> Artificial

<220>  
<223> p74-315 sequence from EcoRV to ATG of GUS

<400> 27  
gatattctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60  
tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatccccg 120  
gggtggtcagt cccttatg 138

<210> 28  
<211> 107  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPUS

<400> 28  
atctccactg acgtaaggga tgacgcaca a tctatatttc aattttattg taatatacta 60  
tataaggaag ttcatttcat ttggagagaa cacgggggac tctagag 107

<210> 29  
<211> 111  
<212> DNA  
<213> Artificial

<220>  
<223> Ros-OPUA

<400> 29  
gatcctctag agtccccgt gttctctcca aatgaaatga acttccttat atagtatatt 60

acaataaaat tgaaatatag attgtgcgtc atcccttacg tcagtggaga t 111

<210> 30  
 <211> 136  
 <212> DNA  
 <213> Artificial

<220>  
 <223> p74-316 sequence from EcoRV to ATG of GUS

<400> 30  
 gatattctcca ctgacgtaag ggatgacgca caatctatat t tcaatttta ttgtaatata 60  
 ctatataagg aagttcattt catttggaga gaacacgggg gactctagag gatccccggg 120  
 tggtcagtcc cttatg 136

<210> 31  
 <211> 108  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ros-OPPS

<400> 31  
 atctccactg acgtaaggga tgacgcacaa tctatatctc aattttattg taatatacta 60  
 tataatatat ttcaatttta ttgtaatata acacggggga ctctagag 108

<210> 32  
 <211> 112  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ros-OPPA

<400> 32  
 gatcctctag agtccc cegt gttatattac aataaaattg aaatatatta tatagtatat 60  
 tacaataaaa ttgaaatata gattgtgcgt catcccttac gtcagtggag at 112

<210> 33  
 <211> 137  
 <212> DNA  
 <213> Artificial

<220>  
 <223> p74-309sequence from EcoRV to ATG of GUS

<400> 33  
 gatattctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60  
 ctatataata tatttcaatt ttattgtaat ataacacggg ggactctaga ggatccccgg 120

gtggtcagtc ccttatg

137

<210> 34  
<211> 237  
<212> DNA  
<213> Artificial

<220>  
<223> p74-118 sequence from EcoRV to ATG of GUS

<400> 34  
gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60  
tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatcctat 120  
atttcaattt tattgtaata tagctatatt tcaattttat tgtaatatata tcgatttoga 180  
acccgggggta ccgaattcct cgagtctaga ggatccccgg gtggtcagtc ccttatg 237

<210> 35  
<211> 235  
<212> DNA  
<213> Artificial

<220>  
<223> p 74-117 sequence from EcoRV to ATG of GUS

<400> 35  
gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatatata 60  
ctatataagg aagttcattt catttgagaga gaacacgggg gactctagag gatcctatat 120  
ttcaatttta ttgtaatatata gctatatattc aattttattg taatataatc gatttcgaac 180  
ccgggggtacc gaattcctcg agtctagagg atccccgggt ggtcagtcctc ttatg 235

<210> 36  
<211> 16  
<212> PRT  
<213> Arabidopsis

<400> 36

Arg Ile Glu Asn Thr Thr Asn Arg Gln Val Thr Phe Cys Lys Arg Arg  
1 5 10 15

<210> 37  
<211> 18  
<212> PRT  
<213> Tobacco

<400> 37

Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser Arg Ile Arg  
1 5 10 15

Lys Lys

<210> 38  
<211> 20  
<212> PRT  
<213> Tobacco

<400> 38

Lys Lys Arg Ala Arg Leu Val As n Arg Glu Ser Ala Gln Leu Ser Arg  
1 5 10 15

Gln Arg Lys Lys  
20

<210> 39  
<211> 18  
<212> PRT  
<213> Maize

<400> 39

Arg Lys Arg Lys Glu Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Ty r  
1 5 10 15

Arg Lys

<210> 40  
<211> 45  
<212> PRT  
<213> Potyvirus

<220>  
<221> MISC\_FEATURE  
<222> (11)..(42)  
<223> where Xaa is any amino acid

<400> 40

Lys Lys Asn Gln Lys His Lys Leu Lys Met Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Arg Lys  
35 40 45

<210> 41  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 41

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys  
1 5 10 15

Ile

<210> 42  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 42

Lys Arg Ile Ala Pro Asp Ser Ala Ser Lys Val Pro Arg Lys Lys Thr  
1 5 10 15

Arg

<210> 43  
<211> 17  
<212> PRT  
<213> Xenopus

<400> 43

Lys Arg Lys Thr Glu Glu Glu Ser Pro Leu Lys Asp Lys Asp Ala Lys  
1 5 10 15

Lys

<210> 44  
<211> 17  
<212> PRT  
<213> Rat

<400> 44

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 45  
<211> 17  
<212> PRT  
<213> Human

<400> 45

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 46  
<211> 17  
<212> PRT  
<213> Human

<400> 46

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 47  
<211> 17  
<212> PRT  
<213> Chicken

<400> 47

Arg Lys Cys Cys Gln Ala Gly Met Val Leu Gly Gly Arg Lys Phe Lys  
1 5 10 15

Lys

<210> 48  
<211> 17  
<212> PRT  
<213> Human

<400> 48

Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Ile Lys  
1 5 10 15

Lys

<210> 49  
 <211> 17  
 <212> PRT  
 <213> Chicken

<400> 49

Arg Arg Cys Phe Glu Val Arg Val Cys Ala Cys Pro Gly Arg Asp Arg  
 1 5 10 15

Lys

<210> 50  
 <211> 236  
 <212> DNA  
 <213> Artificial

<220>  
 <223> p74-114 sequence from EcoRV to ATG of GUS

<400> 50  
 gatatctcca ctgacgtaag ggatgacg ca caatctatat ttcaatttta ttgtaatata 60  
 ctatataata tatttcaatt ttattgtaat ataacacggg ggactctaga ggatcctata 120  
 tttcaatttt attgtaatat agctatatatt caattttatt gtaatataat cgatttcgaa 180  
 cccgggggtac cgaattcctc gagtctagag gatccccggg tggtcagtcc cttatg 236

<210> 51  
 <211> 33  
 <212> DNA  
 <213> Artificial

<220>  
 <223> synRos forward primer

<400> 51  
 gcggatccat gactgagact gcttacggta acg 33

<210> 52  
 <211> 29  
 <212> DNA  
 <213> Artificial

<220>  
 <223> synRos reverse primer

<400> 52  
 gcgagctcga ccttacgctt cttttttgg 29

<210> 53  
 <211> 26



<212> DNA  
<213> Artificial

<220>  
<223> wtRos forward primer

<400> 53  
cgggatccat gacggaaact gcatac 26

<210> 54  
<211> 24  
<212> DNA  
<213> Artificial

<220>  
<223> wtRos reverse primer

<400> 54  
gcgagctcac ggttcgcctt gcgg 24

<210> 55  
<211> 108  
<212> DNA  
<213> Artificial

<220>  
<223> Ros oligonucleotide for Southwestern

<400> 55  
atctccactg acgtaaggga tgacgcacaa tctatatattc aattttattg taatatacta 60  
tataatatat ttcaatttta ttgtaatata acacggggga ctctagag 108

<210> 56  
<211> 43  
<212> DNA  
<213> Artificial

<220>  
<223> Tet oligonucleotide for Southwestern

<400> 56  
gatcactcta tcagtgatag agtgaactct atcagtgata gag 43

<210> 57  
<211> 10  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 57  
tatatttcaa 10

<210> 58  
<211> 10  
<212> DNA

<213> Agrobacterium tumefaciens

<400> 58  
tatattacaa

10

<210> 59  
<211> 10  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 59  
tataattaaa

10

<210> 60  
<211> 10  
<212> DNA  
<213> Agrobacterium tumefaciens

<400> 60  
aatgcgacag

10

<210> 61  
<211> 10  
<212> DNA  
<213> Artificial

<220>  
<223> Ros operator sequence (1)

<400> 61  
tatahttcaa

10